

Environmental Risk Factors for Lyme Disease Identified with Geographic Information Systems

ABSTRACT

Objectives. A geographic information system was used to identify and locate residential environmental risk factors for Lyme disease.

Methods. Data were obtained for 53 environmental variables at the residences of Lyme disease case patients in Baltimore County from 1989 through 1990 and compared with data for randomly selected addresses. A risk model was generated combining the geographic information system with logistic regression analysis. The model was validated by comparing the distribution of cases in 1991 with another group of randomly selected addresses.

Results. In crude analyses, 11 environmental variables were associated with Lyme disease. In adjusted analyses, residence in forested areas (odds ratio [OR] = 3.7, 95% confidence interval [CI] = 1.2, 11.8), on specific soils (OR = 2.1, 95% CI = 1.0, 4.4), and in two regions of the county (OR = 3.5, 95% CI = 1.6, 7.4) (OR = 2.8, 95% CI = 1.0, 7.7) was associated with elevated risk of getting Lyme disease. Residence in highly developed regions was protective (OR = 0.3, 95% CI = 0.1, 1.0). The risk of Lyme disease in 1991 increased with risk categories defined from the 1989 through 1990 data.

Conclusions. Combining a geographic information system with epidemiologic methods can be used to rapidly identify risk factors of zoonotic disease over large areas. (*Am J Public Health.* 1995;85:944-948)

Gregory E. Glass, PhD, Brian S. Schwartz, MD, MS, John M. Morgan III, PhD, Dale T. Johnson, MA, Peter M. Noy, and Ebenezer Israel, MD

Introduction

Lyme disease, caused by *Borrelia burgdorferi*, is the most frequent vector-borne disease in the United States.¹ Areas such as woodland and forest-edge habitats that support large numbers of vectors and mammalian hosts of *B. burgdorferi* are considered high-risk areas for disease.² However, place of residence has been recognized as a risk factor, and attention has been focused on the acquisition of disease in and around the residence.^{3,4} Investigators have attempted to identify high-risk areas for Lyme disease by techniques such as surveying for tick vectors and estimating host population abundances.⁵⁻⁸ Because of the time, effort, and money involved in surveying vector and host populations,⁹ identifying easily measured environmental factors that are surrogates of risk would improve our ability to assess risk over larger areas.

Several workers have attempted to identify environmental characteristics of areas associated with *Ixodes* populations as a measure of risk. Schulze and colleagues devised an index of risk for Lyme disease in New Jersey.¹⁰ Using surveys of habitat throughout New Jersey, they identified environmental characteristics associated with *Ixodes scapularis*, including habitat suitability, size, and accessibility. *I. scapularis* were abundant in areas with a mixture of hardwood and conifer trees that had layers of shrubby vegetation, such as forest edges.¹¹ Tick abundance also has been associated with humidity, temperature, slope of the landscape,¹² forested areas with sandy soils,¹³ and the intensity of residential development.⁴ There is a need to extend risk analysis to larger, less well defined areas while reducing the expenditure of time and resources.

The development of geographic information systems during the past 20 years provided the impetus for geographers to analyze large-scale spatial patterns.¹⁴ Consequently, many detailed environmental databases have been developed by federal, state, and local agencies. The present study combined a geographic information system, geographic data, and case-control epidemiologic methods to identify environmental risk factors for Lyme disease in Baltimore County, Md, a 1560-km² area.

Methods

Study Site

Baltimore County, Md, lies on the northwestern shore of the Chesapeake Bay, including regions of both Coastal Plain and Piedmont. The county had a population of 692 134 in 1990.¹⁵ Approxi-

Gregory E. Glass is with the Department of Molecular Microbiology and Immunology, and Brian S. Schwartz is with the Department of Environmental Health Sciences and the Department of Epidemiology, The Johns Hopkins University School of Hygiene and Public Health, Baltimore, Md. John M. Morgan III is with the Department of Geography and Environmental Planning, Towson State University, Towson, Md. Dale T. Johnson is with the Baltimore County Department of Environmental Protection and Resource Management, Towson, Md. Peter M. Noy is with the Baltimore County Office of Planning and Zoning, Towson, Md. Ebenezer Israel is with the Maryland State Department of Health and Mental Hygiene, Baltimore, Md.

Requests for reprints should be sent to Gregory E. Glass, PhD, Department of Molecular Microbiology and Immunology, The Johns Hopkins University School of Hygiene and Public Health, 615 N Wolfe St, Baltimore, MD 21205.

This paper was accepted October 11, 1994.

TABLE 1—Crude and Adjusted Odds Ratios (ORs) for Environmental Variables Associated with Lyme Disease in Baltimore County, Maryland, 1989 through 1990

	Crude OR (95% CI)	Adjusted OR ^a (95% CI)
Watersheds^b		
Little/Lower Gunpowder vs reference	3.0 (1.0, 8.7)	2.8 (1.0, 7.7)
Pretty Boy/Loch Raven vs reference	4.4 (2.1, 9.4)	3.5 (1.6, 7.4)
Land use^b		
Highly developed vs reference	0.3 (0.1, 1.4)	0.3 (0.1, 1.0)
Soils^b		
Fair—good conifer habitat vs poor	2.5 (1.2, 5.2)	
Poor—fair herbaceous habitat vs good	1.9 (1.0, 3.7)	
Manor soils ^c vs reference	3.4 (1.3, 8.9)	
Glenville/Glenelg soils ^c vs reference	2.8 (1.3, 5.9)	2.1 (1.0, 4.4)
Geology^b		
Loch Raven schist vs reference	5.9 (2.2, 15.9)	
Combined geology vs other (Cockeysville marble/Baltimore gneiss/Slaughterhouse gneiss)	5.7 (2.5, 13.0)	

^aAdjusted for variables in the final logistic regression model (Glenville/Glenelg soils, high-density development, Pretty Boy/Loch Raven watersheds, Little/Lower Gunpowder watersheds, forested areas). Variables without values were not included in the final model.

^bOriginal database used for extracting environmental variables. Only variables with significant associations with Lyme disease are included.

^cThe name given to this particular soil series by the US Department of Agriculture.

mately 16% of the area is heavily developed, 25% is devoted to agriculture, 39% is residential, 10% is designated as parks, and 11% is covered by fresh water. Thirty percent of the county is covered by deciduous or mixed coniferous and deciduous forests. Baltimore County was selected for study because it currently has one of the best developed sets of environmental databases within the state, and it had the largest number of cases of Lyme disease in the state during 1989 and 1990.

Study Subjects

Lyme disease was first reported in Maryland in 1979, and incident cases increased to 237 by 1990.¹⁶ It became a reportable disease in the state in 1989. Baltimore County recorded the largest number of cases in the state¹⁷ in 1989 and 1990.

For this study, case patients were defined as Baltimore County residents reported to the state health department and subsequently confirmed to meet the Centers for Disease Control case definition during 1989 and 1990 ($n = 47$).¹⁷ As controls, 492 residences were randomly selected from a database of address records for the county maintained by the Baltimore County Department of Environmental Protection and Resource Management.

To validate the model, address information for the residences of patients with

incident Lyme disease cases in 1991 ($n = 48$) was obtained, and 495 additional addresses were randomly selected. Addresses of both case patients and control subjects were recorded as state plane coordinates to geographically locate the residences.

Study Variables

Information was obtained on the patient's residence and the geographic location where the tick bite occurred, if known. Six patients reported tick bites away from their residences.

Six databases were used to obtain environmental information. These were land use/land cover, forest distributions, soils, elevation, geology, and watershed databases maintained by the Baltimore County Department of Environmental Protection and Resource Management and the Department of Geography, Towson State University. All databases had a 400 ft \times 500 ft (121.9 m \times 152.4 m) resolution, producing a grid map of the county with 164 248 cells for each variable.

The land use/land cover database was generated from a composite of a LANDSAT Thematic Mapper satellite image obtained in 1990 and from planimetric maps of the urban portions of the county.¹⁸ The satellite image had a pixel resolution size of 30 m \times 30 m, and the

TABLE 2—Sample Sizes for Environmental Variables Associated with Lyme Disease in Baltimore County, Maryland, 1989 through 1990

	No. Case Patients	No. Control Subjects
Watersheds^a		
Little/Lower Gunpowder	6	29
Reference	21	306
Pretty Boy/Loch Raven	17	56
Reference	21	306
Land use^a		
Highly developed	3	102
Reference	11	103
Soils^a		
Fair—good conifer habitat	13	71
Poor	34	421
Poor—fair herbaceous habitat	19	134
Good	28	358
Manor soils ^b	8	33
Reference	24	326
Glenville/Glenelg soils ^b	15	76
Reference	24	326
Geology^a		
Loch Raven schist	11	56
Reference	10	300
Combined geology	26	137
Reference	10	300

^aOriginal database used for extracting environmental variables. Only variables with significant associations with Lyme disease are included.

^bThe name given to this particular soil series by the US Department of Agriculture.

planimetric maps had a scale of 1:2400. The land use/land cover database used three categories of residential property, six categories of urban property, three categories of zoned but undeveloped residential land, four categories of zoned but undeveloped urban property, and one category each of agricultural land, recreational land, forested land, and water.

The forest distribution database was generated from the same LANDSAT image by using an unsupervised classifica-

TABLE 3—Linear Trends for the Associations of Distance from Forest and Land Slope with Lyme Disease Risk at the Place of Residence

	Crude OR (95% CI)	Adjusted OR ^a (95% CI)
Distance from forest, ft ^b		
0	9.0 (2.8, 29.8)	3.7 (1.2, 11.8)
> 0–640	3.0 (1.0, 9.4)	1.5 (0.5, 4.5)
641–1300	1.8 (0.6, 5.8)	1.1 (0.4, 3.4)
1301–2600	1.2 (0.4, 4.2)	0.9 (0.3, 2.9)
> 2600 ^c	1.0	1.0
Slope, degrees ^d		
0 ^c	1.0	1.0
0.26–1.00	1.5 (0.5, 4.7)	1.3 (0.4, 3.6)
1.01–2.30	2.0 (0.7, 5.7)	1.2 (0.4, 3.3)
> 2.30	3.5 (1.4, 9.6)	1.6 (0.6, 4.4)

^aVariables in the final logistic regression model are listed in Table 1.

^bTest for linear trend for crude association: $\chi^2 = 14.50$; $P = .0001$.

^cReference category.

^dTest for linear trend for crude association: $\chi^2 = 9.06$; $P = .0026$.

The soils database was encoded from the US Department of Agriculture soil survey database for Baltimore County with a 1-acre (0.004-km²) resolution.²⁰ The soils database mapped 61 soil series recognized in the county.

The geology database was digitized from Maryland Geological Survey maps with scales of 1:63 360. It contained the 30 rock formations within the county. The watershed database was digitized from 1:63 360 maps maintained by the Baltimore City Department of Public Works and showed the distributions of the 15 watersheds.

Geographic Information System

Environmental databases and the state plane coordinates of the residences of case patients and control subjects were imported into IDRISI, a raster-based geographic information system developed by Clark University Graduate School of Geography.²¹ The original environmental databases were manipulated with resident software procedures to extract the study variables that were used for data analysis. Initially, 127 study variables were extracted from the databases. The variables were evaluated at the residence of each case patient and control subject.

Data Analysis

Analyses were performed with the BMDP statistical software system.²² Most variables were dichotomized, and those ($n = 74$) with small marginal sample sizes were excluded from further analyses unless they were spatially contiguous and shared physical properties (e.g., two adjacent soil types with similar soil chemistries and physical properties), in which case they were combined. Crude odds ratios (ORs) were calculated for the associations between study variables and Lyme disease. Fisher's exact P values were calculated when expected cell sizes were less than 5.

Continuous variables (elevation, aspect, slope, distances to parks and forests) were categorized by quartiles, and linear trends were evaluated by the chi-square test. Stratified analysis and logistic regression were used to control for confounding variables. Variables that were thought to be important a priori or that were statistically significant in the crude analyses were included in the initial logistic regression model, and their contributions were assessed by partial F tests. The remaining variables were added to this initial model, and their contributions also were evaluated by partial F tests. After

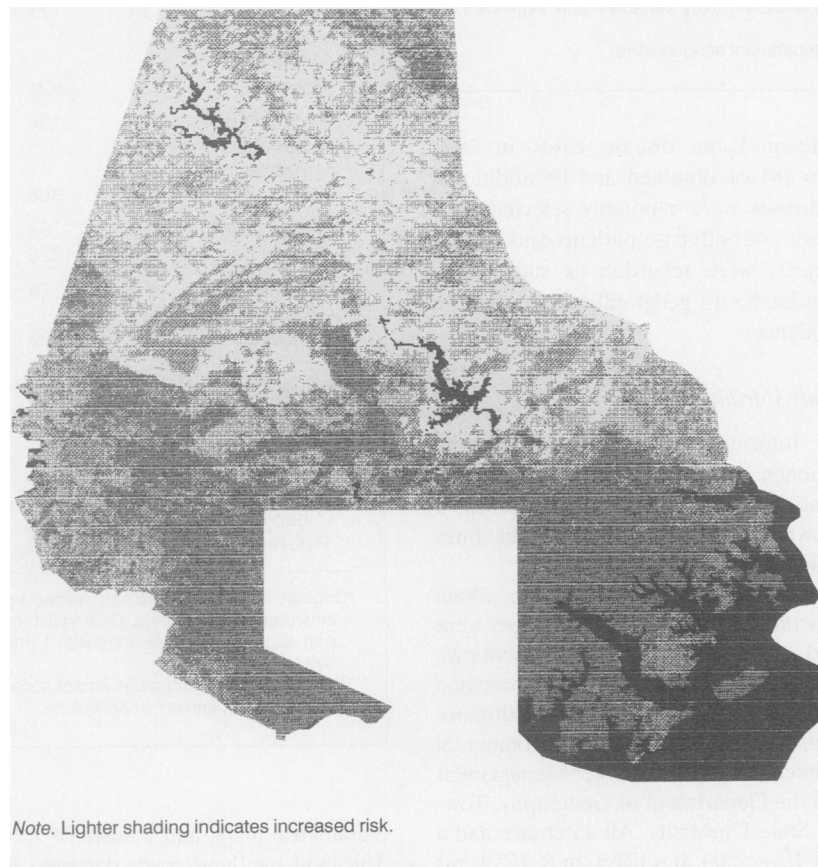


FIGURE 1—Lyme disease—risk density map from logistic regression model that used environmental variables in Baltimore County, Maryland, 1989 through 1990.

tion procedure.¹⁹ A cell was classified as forest if more than half the cell was forested. This database differed from the forested-land category in the land use/

land cover database because it incorporated recreational areas as well as some agricultural and low-density residential areas.

selection of the final model, all possible interactions between variables were assessed and retained when statistically significant ($P < .05$).

In 1989 through 1990, six cases were possibly misclassified as to their site of tick exposure. The analyses were repeated after removing these six cases from the analysis. This final logistic regression model did not differ from the model with the six cases included, so only the model with all cases is shown. Data on locations of tick exposures were not available in 1991.

Lyme Disease Map

The logistic regression equation was used to generate a map indicating the areas of the county where Lyme disease was most likely to occur. The logistic function was imported into IDRISI, and the maps associated with the variables in the final model were used to generate a composite map of risk.²³ The scores were grouped into quartiles for display purposes. Areas of the county falling into the various risk categories were determined with procedures resident in IDRISI. The model was validated by calculating the Hosmer–Lemeshow goodness-of-fit statistic (C) for the 1989 through 1990 data and by comparing trends in disease risk in 1991 with risk as defined by the analysis of the 1989 through 1990 data.

Results

Residences of Lyme disease case patients in Baltimore County in 1989 and 1990 extended in a northwest to southeast line through the county, with most clustering around a reservoir in the north-central portion of the county.²⁴ A second, smaller cluster occurred in the southwest portion of the county. Control addresses clustered in areas of high population density in three groups, along the east, west, and north Baltimore City–Baltimore County boundaries.

Eleven (21%) of the environmental variables extracted from the databases were associated ($P < .05$) with Lyme disease (Tables 1–3). The risk of disease decreased with increasing distance from forest edge and increased with increasing steepness of slope (Table 3). These trends also were apparent in adjusted analyses, although less dramatically (Table 3). Distances from streams and parks were not associated with a change in risk ($P > .40$). There was a trend for risk to

TABLE 4—Linear Trends for the Association of Lyme Disease in 1991 with Lyme Disease Risk at the Place of Residence in 1989 through 1990, the Areal Extent of Baltimore County in Each Category, and the Estimated Incidence of Disease

Risk Category 1989 through 1990 ^a	OR (95% CI)	Area, km ²	1991 Incidence (per 100 000)
Lowest ^b	1.0	486	4.35
Low	2.2 (1.0, 4.8)	403	9.54
Moderate	3.2 (1.2, 8.6)	374	13.91
High	16.4 (4.7, 58.5)	296	71.52

^aTest for linear trend for association: $\chi^2 = 29.10$; $P < .00001$.

^bReference category.

increase with altitude ($\chi^2 = 2.98$; $P = .08$). However, risk decreased at the highest altitude.

Among categorical variables, risk of Lyme disease was elevated in two adjacent northern watersheds (Pretty Boy and Loch Raven) (Table 1). Highly developed areas, such as multiunit residential neighborhoods, tended to be associated with a reduced risk of disease (Table 1). Soils that were suitable for conifers or were unsuitable for herbaceous vegetation tended to be associated with an increased risk of disease. Well-drained, deep, loamy soils in the Piedmont also represented a risk for disease. Certain underlying geological formations from which the soil developed also were associated with increased risk (Table 1).

Five variables met the criterion for inclusion in the final logistic regression model. Residence within the combined Pretty Boy and Loch Raven watersheds, within the combined Little and Lower Gunpowder watersheds, in forested areas, and on deep, loamy soils in the Piedmont was associated with increased risk, whereas residence in highly developed areas was a protective factor (Table 1). There tended to be an interaction between the presence of loamy soils and the presence of forests around the residence. The odds ratio associated with residence in a forested area was 6.8 (95% confidence interval [CI] = 2.4, 19.3) when loamy soil was present, but it was only 1.8 (95% CI = 0.4, 8.5) when loamy soil was absent (P value for interaction = .16). The model fit the 1989 through 1990 data well ($C = 4.23$, $df = 8$, $P = .84$).

The final logistic regression model was used to generate a map of Lyme disease risk for the county (Figure 1). Overall, 19.0% of the county was classified in the highest-risk category for Lyme disease, and 31.2% was classified in the

lowest-risk category (Table 4). A total of 25.9% of the county was in the low quartile and 24.0% in the moderate quartile.

The pattern of risk identified from the 1989 through 1990 data was maintained the following year. In 1991 the gradient of risk across the three highest quartiles increased exponentially ($\chi^2 = 29.1$; $P < .00001$) (Table 4) relative to the lowest-risk areas. The odds of infection associated with residence in low-risk areas was marginally elevated over that in the reference category (OR = 2.2, 95% CI = 1.0, 4.8), whereas residence in the highest-risk areas was associated with a marked increase in the odds of infection (OR = 16.5, 95% CI = 4.7, 58.5). Overall, 85.8% of the 1991 case patients and control subjects were correctly classified by the 1989 through 1990 map. Based on the distribution of the 1991 random residences, we estimate that 9788 people resided in the highest-risk areas. This suggests an incidence rate of 72/100 000 residents per year in these areas, compared with a rate of 4/100 000 in the lowest-risk areas (Table 4).

Discussion

This study demonstrates that a geographic information system may be useful in identifying environmental risk factors associated with vector-borne infectious diseases. Combining a geographic information system with epidemiologic analysis allows us to study the spatial patterns of disease over larger geographic areas with greater accuracy than was previously possible.

Current approaches in geographic information systems tend to focus on characterizing the spatial distribution of points or areas in relation to one another.

er.²¹ For example, Kitron and colleagues examined the spatial distribution of capture locations of *I. scapularis*-infested deer in northern Illinois.¹³ Observational epidemiologic methods have not been used in geographic information system studies to analyze spatial data. Yet these methods may offer substantial advantages in analyzing data derived from geographic information systems.

Early studies suggested that residence was a risk factor for Lyme disease, although the basis for the association was not established.²⁵ Subsequently, Maupin and colleagues showed that substantial numbers of *I. scapularis* occurred in and around residences.⁴ Residence was chosen for the present analysis because most case patients (87%) identified no other area for likely tick exposure.

Eliminating case patients who were exposed to the disease away from their residences did not affect the spatial pattern of the risk map. However, in 1989 through 1990, five of these six case patients resided in the lowest-risk areas, suggesting that the detailed epidemiologic analyses may be useful in reducing misclassification. Our results indicate that the environmental conditions in the vicinity of residence are important, easily characterized measures of Lyme disease risk.

The environmental variables in the logistic regression model were consistent with the epidemiology of Lyme disease. The risk associated with residence in forested areas has been recognized since the earliest reports of Lyme disease,²⁵ presumably because of the habitat requirements of both the vector and the reservoir species.²⁶ The protective nature of high-development areas is linked to the inverse association between vector abundance and the intensity of development in residential areas.⁴

The significant association with loamy soils also appears related to vector abundance.²⁷ These loams are mixtures of predominantly sandy soils, with additions of silt and clay.²⁰ Kitron and colleagues reported that *I. scapularis* were most abundant on sandy soils with deciduous forests in Illinois.¹³

The large number of study variables relative to the number of cases raises the possibility of chance findings. Before the results of this study can be generalized to other geographic areas, the associations of human disease with soil type should be replicated in other regions.

The increased risk of Lyme disease within specific watersheds may represent

surrogate measures of host abundance for adult ticks.⁷ The four watersheds with an increased odds of Lyme disease are among those with the densest deer populations in the county (Maryland Department of Natural Resources, 1992, unpublished data).

A major consideration in the study of zoonotic diseases is determining where control and prevention measures should be focused. Uniting epidemiologic methods, which identify factors associated with disease, with geographic information system methods, which determine where these factors occur, provides an approach to study the distribution of diseases that are influenced by a multitude of environmental factors. □

Acknowledgments

This research was supported by grants from the National Institute of Allergy and Infectious Diseases (R55 AI30042 and R29 AI31608).

References

- Centers for Disease Control. Lyme disease—United States, 1991–1992. *MMWR Morb Mortal Wkly Rep.* 1993;42:345–348.
- Steere AC. Lyme disease. *N Engl J Med* 1989;321:586–596.
- Falco RC, Fish D. Prevalence of *Ixodes dammini* near the homes of Lyme disease patients in Westchester County, New York. *Am J Public Health.* 1988;127:826–830.
- Maupin GO, Fish D, Zultowsky J, Campos EG, Piesman J. Landscape ecology of Lyme disease in a residential area of Westchester county, New York. *Am J Epidemiol.* 1991;133:1105–1113.
- Mather TN, Wilson ML, Moore SI, Ribeiro JMC, Spielman A. Comparing the relative potential of rodents as reservoirs of the Lyme disease spirochete (*Borrelia burgdorferi*). *Am J Epidemiol.* 1989;130:143–150.
- Anderson JF, Johnson RC, Magnarelli LA, et al. Identification of endemic foci of Lyme disease: isolation of *Borrelia burgdorferi* from feral rodents and ticks (*Dermacentor variabilis*). *J Clin Microbiol.* 1985;22:36–38.
- Wilson ML, Ducey AM, Litwin TS, et al. Microgeographic distribution of immature *Ixodes dammini* ticks correlated with that of deer. *Med Vet Entomol.* 1990;4:151–159.
- Wilson ML, Adler GH, Spielman A. Correlation between abundance of deer and that of the deer tick, *Ixodes dammini* (Acari: Ixodidae). *Ann Entomol Soc Am.* 1986;79:172–176.
- Piesman J, Mather TN, Dammin GJ, et al. Seasonal variation of transmission risk of Lyme disease and human babesiosis: the authors reply. *Am J Epidemiol.* 1988;128:1383–1384.
- Schulze TL, Taylor RC, Taylor GC, Bosler EM. Lyme disease: a proposed ecological index to assess areas of risk in the northeastern United States. *Am J Public Health.* 1991;81:714–718.
- Schulze TL, Lakat MF, Bowen GS, Parkin WE, Shisler JK. *Ixodes dammini* (Acari: Ixodidae) and associated ixodid ticks collected from white-tailed deer in New Jersey, USA: geographical distribution and relation to selected environmental and physical parameters. *J Med Entomol.* 1984;21:741–749.
- Lane RS, Stubbs HA. Host-seeking behavior of adult *Ixodes pacificus* (Acari: Ixodidae) as determined by flagging vegetation. *J Med Entomol.* 1990;27:282–287.
- Kitron U, Jones CJ, Bouseman JK, Nelson JA, Baumgartner DL. Spatial analysis of the distribution of *Ixodes dammini* (Acari: Ixodidae) on white-tailed deer in Ogle county, Illinois. *J Med Entomol.* 1992;29:259–266.
- Aronoff S. *Geographic Information Systems: A Management Perspective.* Ottawa, Canada: WDL Publications; 1989.
- General Population Characteristics.* Washington, DC: Bureau of the Census; 1990.
- Amerasinghe FP, Breisch NL, Azad AF, et al. Distribution, density, and Lyme disease spirochete infection in *Ixodes dammini* (Acari: Ixodidae) on white-tailed deer in Maryland. *J Med Entomol.* 1992;29:54–61.
- Mitchell CS, Cloeren M, Israel E, Lazar C, Schwartz BS. Lyme disease in Maryland, 1987–1990. *Maryland Med J.* 1992;41:391–396.
- Noy PM, Johnson DT. Combining GIS and remotely sensed data: a case study of the development of a county land use map. *TSU GIS 92.* 1992;12.
- Jensen JR. *Introductory Digital Image Processing: A Remote Sensing Perspective.* New York, NY: Prentice Hall Inc; 1986.
- Soil Survey: Baltimore County, Maryland.* Washington, DC: US Dept of Agriculture; 1976.
- Eastman JR. *IDRISI: A Grid-Based Geographic Analysis System, Version 4.0 Edition.* Worcester, Mass: Clark University Graduate School of Geography; 1992.
- Dixon WJ, ed. *BMDP Statistical Software Manual.* Vols I and II. Berkeley, Calif: University of California Press; 1988.
- Chatterjee S, Price B. *Regression Analysis by Example.* New York, NY: John Wiley & Sons Inc; 1977.
- Glass GE, Morgan JM III, Johnson DT, Noy PM, Israel E, Schwartz BS. Infectious disease epidemiology and GIS: a case study of Lyme disease. *Geo Info Systems.* 1992;2(November-December):65–69.
- Steere AC, Malawista SE, Snyderman DR, et al. Lyme arthritis: an epidemic of oligoarticular arthritis in children and adults in three Connecticut communities. *Arthritis Rheum.* 1977;20:7–17.
- Spielman A, Wilson ML, Levine JE, Piesman J. Ecology of *Ixodes dammini* borne human babesiosis and Lyme disease. *Annu Rev Entomol.* 1985;30:439–460.
- Glass GE, Amersinghe FP, Morgan JM, Scott TW. Predicting *Ixodes scapularis* abundance on white-tailed deer using geographic information systems. *Am J Trop Med Hyg.* 1994;51:538–544.